STARVATION STUDIES WITH THE PARASITIC WASP HABROBRACON

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Introduction

The present investigation on the braconid known in genetic literature as *Habrobracon juglandis* (Ashmead) suggested itself as a test of the hypothesis that the sex difference in fat cell size (Grosch, 1948c) may be interpreted on the basis of single versus double storage of reserves. That is, females are suspected to possess, in the form of ovaries, an additional and dominant storage tissue, competing in activity and mass for space in the confines of the wasp abdomen.

Furthermore if utilization of eggs occurs similar to that described for other Hymenoptera (Flanders, 1938, 1942a, 1942b) it is desirable to determine when the process begins and to what extent it proceeds, because basic techniques of important investigations on the effects of irradiation involve Habrobracon egg production (A. R. Whiting, 1949; G. A. Heidenthal, 1945).

Finally, as reference for proposed investigations on morphogenesis, it was desirable to know the size relationships of major internal structures under the normal and the most extreme conditions of the laboratory environment.

Part I: Investigations at Incubator Temperature

Because a majority of experimental rearing of Habrobracon is conducted under incubation, attention was especially directed to this environment.

Materials and methods. Wild type stock 33 provided the normal animal types used, namely impaternate males and biparental females. Since the purpose was to investigate the organism under routine laboratory conditions, no exceptional provisions for humidity control were made. Open pans (10 × 8 × 2 in.) of water were (and are) maintained on the lowest shelf of the 28 × 16 × 28 in. incubator. Temperature was maintained at 30–35° C. with maximum-minimum thermometer keeping the record. Shell vials (70 × 20 mm.), plugged with cotton (enclosed in cheesecloth), were the containers employed in stock maintainance and for all experiments. Daily examinations were made of all vials. Thus day of emergence from cocoon and day of death were obtained.

Since not even water was given to the animals under investigation, the "most extreme" type of routine laboratory environment was provided. Incidentally, results in a small scale exploratory experiment had shown average mortality to occur slightly earlier in groups provided with water, which probably is explainable by a tendancy of weakened individuals to blunder into the water and drown. This observation is consistent with Genieys' (1925) statement that mortality is very great when starving braconids are given nothing besides pure water.

For purposes of estimating the site of the major loss of substance during starvation, wasps were transected at the petiole and the weights of abdomens and weights of anterior regions were compared in freshly eclosed and in starved wasps.

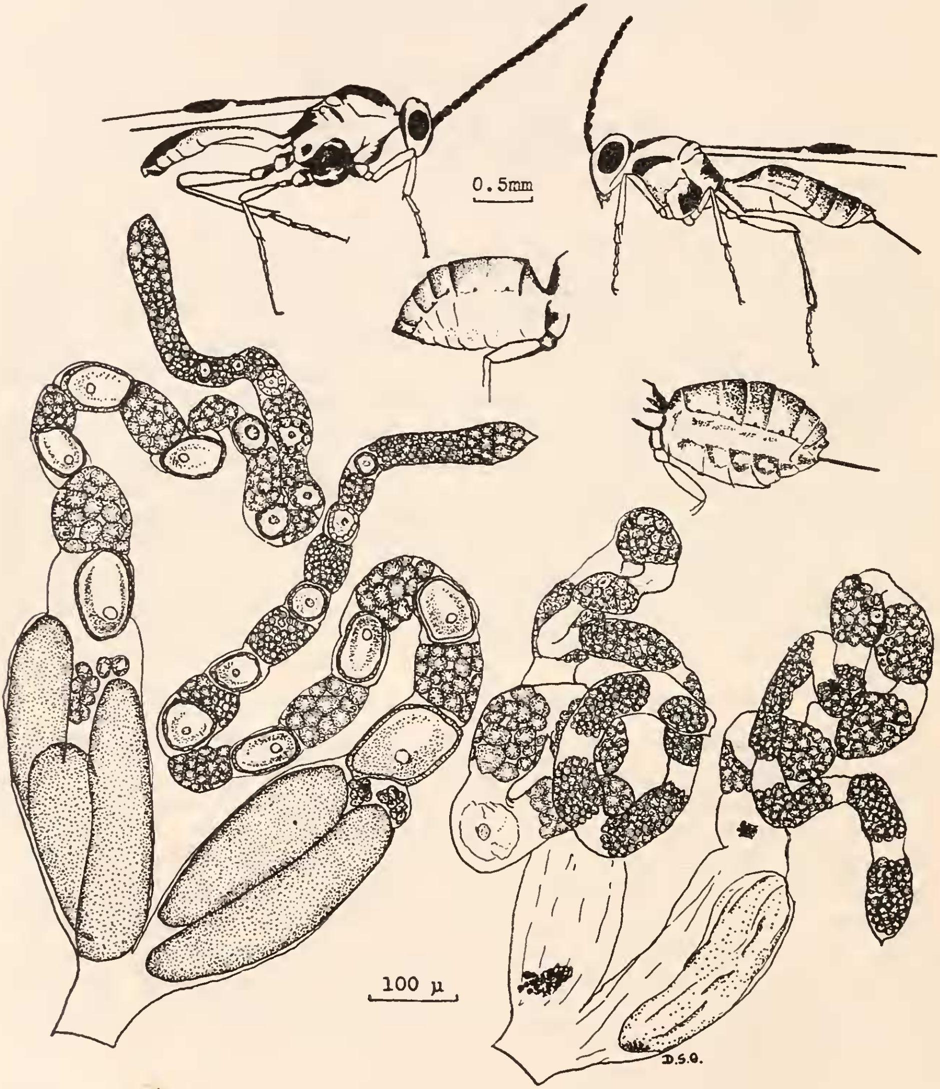

FIGURE 1. Above: Lateral views of a starved male (Left) and a starved female (Right) with abdomens of an unstarved male and female for comparison. The extreme dorsoventral flattening depicted can be seen in starved animals which are still quite active. It is interesting to note how this condition approximates the appearance of pinned museum specimens. Below: Ovarioles, each pair of which constitutes one of the two ovaries of (Left) an unstarved female recently emerged from her cocoon and (Right) a starved female dissected in what would have been the last or second last day of her life. The empty areas in the latter represent locations from which developing ova have disappeared.

For structural measurements wasps in various stages of starvation were dissected under a binocular microscope and the internal organs immediately fixed with 10 per cent formalin. This procedure was adopted because of the contrasts the fixative quickly develops in the ovaries and accessories. The preparation was then examined under a compound microscope and measurements of tissue elements taken by means of an ocular micrometer. The series of observations was continued until data was available for at least 5 wasps of each sex for each day during the starvation period.

Results

Longevity. The survival time in days as given by the mean and its standard error was 6.13 ± .16 for 100 males and 6.68 ± .30 for 100 females. All animals comprising these samples were within the normal size range and no correlation between the size of animal and the survival time in days was apparent (correlation constant $r = 0$). Measured from the time of eclosion the earliest deaths occurred on day 3, oldest survivors expired on day 10.

Weight loss. Comparisons of transected wasps indicate that weight is lost chiefly from the abdomen. For 10 males of selected size (2.35 to 2.47 mm. long) the anterior parts comprising the head and thorax-with-appendages averaged (mean with its standard error) .47 ± .04 mg. at eclosion, and .45 ± .04 after starvation; while the abdomens averaged .52 ± .05 mg. at eclosion and .38 ± .06 mg. after starvation. For 10 equivalent females, anterior parts averaged .50 ± .06 mg. at eclosion and .50 ± .05 mg. after starvation, while abdomens averaged .82 ± .07 mg. at eclosion and .38 ± .06 mg. after starvation. Weight losses from the abdomen are accompanied by dorso-ventral flattening which becomes extreme in the last two days of life (see Fig. 1). In fact, in this dimension the male abdomen becomes so thin it actually approaches translucence, with shadowed areas indicating the positions of the digestive tract and gonads.

Histological changes. Presented in Table I are measurements of internal structural units in unstarved (0 hour, 0 day; at eclosion) and starved wasps (unable to stand; therefore in the last day of life). Ten animals from each class are represented by this summarization. A summary of the series of examinations of internal structures made at daily intervals is untabulated because it merely provides values intermediate between the extremes of Table I. It is evident that the perceptible changes accompanying weight loss occur in the abdominal contents and especially in the "fat" cells. When the animal is adult this type of cell is found only in the abdomen (Grosch, 1949). In reference to the fat cells there is an additional untabulated observation on the abdominal contents of starved animals. That is, it is difficult to find 100 fat cells for measurement after starvation in contrast to conditions in unstarved wasps where there are typically several hundred fat cells. On the other hand there seems to be no significant change in number of urate cells as shown by the following means and standard errors: for unstarved males 35.20 ± 4.10, for starved males 31.25 ± 4.25, for unstarved females 34.33 ± .27, for starved females 31.16 ± 3.50.

Conspicuously absent in Table I are measurements of the ovarioles. This is due to the fact that because of contortion in starved animals, length could not be determined. However, diameters, as shown in the accompanying drawing (Fig. 1), change but little in ovarioles compared from starved and unstarved females. The change which is very apparent in comparisons is the effect of a sorptive process. As seen, this has involved not only mature eggs but also developing eggs. Following these events in daily dissection, it is during the fourth day at incubator

TABLE I

Means and standard errors to represent dimensions in microns of internal structures of wasps 2.35 mm. to 2.47 mm. in length

Abdominal contents	Males		Females	
	Unstarved	Starved	Unstarved	Starved
Urate cell diameter	161.15± 2.64	110.22± 2.64	128.48±2 1.98	105.49± 3.19
Fat cell diameter	141.02± 1.87	89.10± 1.43	132.33± 1.43	78.21± 1.32
Crop				
Length	728.42±27.17	725.78±32.45	715.00±111.65	1005.40±119.02
Width	308.00±23.98	297.00±23.87	244.75± 44.11	294.80± 34.76
Stomach				
Length	889.13±14.41	880.00± 2.86	1031.25± 72.82	1086.25± 83.38
Width	330.00±27.50	253.00±14.74	481.25± 28.49	379.50± 33.99
Hindgut				
Length	891.00±33.60	861.63± 9.46	916.63± 15.18	941.75± 24.86
Width*	180.40± 9.57	190.63±15.62	231.00± 28.82	195.25± 4.95
Malpigh. tube				
Length	1353.00±60.39	1251.25±22.88	1375.00± 47.74	1320.00± 33.66
Width	33.00± 5.00	33.00± 1.65	33.00± 5.30	31.79± 8.80
Testis				
Length	270.60± 5.17	214.50± 4.18		
Width	184.80± 2.75	144.00± 1.75		
Accessory tube				
Length	371.25±22.77	368.50±26.07		
Width	74.25± 4.29	79.75± 4.29		
Abdominal ganglion (2nd last)				
Length	49.00± .55	49.00± .55	89.10± .55	93.50± 7.38
Width	49.00± .55	49.00± .55	91.63± 1.65	93.50± 8.69
Poison gland				
Length			770.00± 44.66	715.00± 27.50
Width			90.75± 8.36	107.25± 10.23
Reservoir				
Length			291.50± 11.88	253.00± 13.64
Width			17.05± .28	13.75± 7.26
Thoracic muscles diameters				
Dorsal long.	94.60± 3.85	96.80± 4.73	101.20± 4.73	96.80± 4.84
Alars	60.94± 1.54	59.40± .77	53.13± 1.54	50.95± 1.54
Promotors and remotors	25.19± .22	22.00± 2.09	22.00± 1.87	20.13± 1.65

* This dimension is given for the broadest part, the narrowest part in both males and females is about 77 μ in diameter.

temperature that the egg contents of the ovarioles are visibly depleted. To day 3 inclusive, a total of 10 to 12 full sized eggs can be found for the four egg sacs. The contents of both the mature and the developing ova appear to be quantitatively adequate during this period. After the third day changes are visibly apparent,

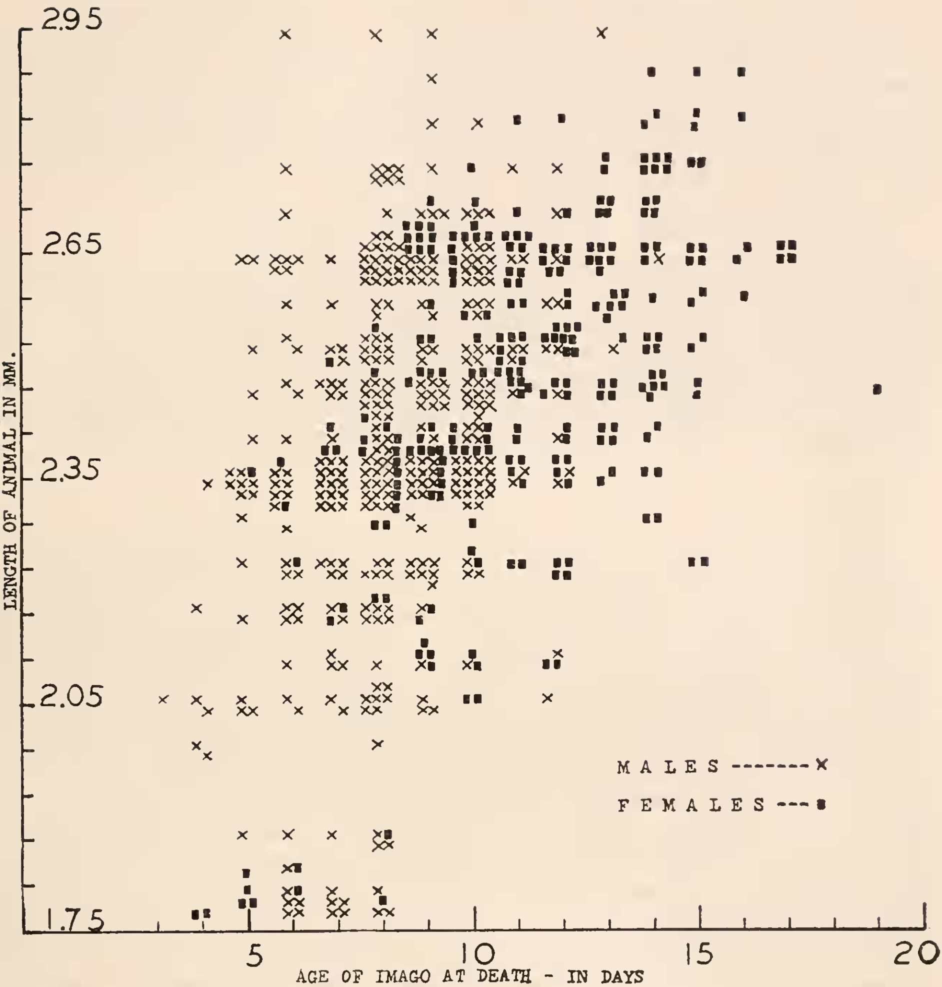

FIGURE 2. Scatter diagram showing the relative longevity of starved males and females of the sizes obtained in routine culture of Habrobracon. Notable is the tendency of females to live longer than males of equal size as reflected in the propensity of the squares to lie more to the right than the x marks.

especially in lessened opacity of egg regions. After five days of starvation no more than two eggs have been found in any dissected abdomen and after six days no full sized eggs have been found. On the other hand, there are no visible signs of sorption of developing eggs until a day or two before death (day 8 or 9 at incubator temperature). Incidentally, the relatively slight but definite change in testis size (Table I) also is not demonstrable until the last two days before death. In general these oösorption events have striking similarities to the observations on the honey bee and ants mentioned by Flanders (1942b) as being made first by Weyer.

Part II: Longevity at Room and Refrigerator Temperatures

When it is desired to slow up development and increase the period of time between the transfers necessary for stock maintainance, common laboratory practice is to keep Habrobracon vials at room temperature. Another routine procedure is the storage of excess wasps in a refrigerator. In order to investigate an impression that females were the last survivors in vials kept under these conditions, specific observations were made on samples of wasps of known age and size.

Materials and methods. Wild type stock 33 again provided the wasps which were stored in clean shell vials. Death by causes other than starvation had been observed to accompany storage in vials containing host caterpillars, inviable pupae and other miscellaneous debris. Room storage at temperature 20–25° C. was investigated in the summertime at the Marine Biological Laboratory, Woods Hole, Massachusetts where humidity is generally high (typical relative humidity is between 70–80%). Vials stored in an electric refrigerator at 4–5° C. were placed in a closed metal container around an open wide-mouth bottle full of water.

Results

Room temperature. At 20–25° C. a distinct positive correlation is evident between animal size (as represented by a measurement such as length) and longevity (as given by day of death). Correlation constants (r) were calculated as .3404 for males and .5746 for females. (Thanks is expressed to Dr. H. F. Robinson and technical assistants of the N. C. State College Dept. of Experimental Statistics for computing this pair of values.) Furthermore, the adult life of males was significantly shorter (7.98 ± .02 days) than that of females (11.27 ± .02 days) as measured by calculating mean survival time and standard error. A graphic picture of these facts is given by the scatter diagram Figure 2. An analysis also was made of these same data from the standpoint of number of individuals per vial. This comparison of viability in experiments when less than 10 (average = 5) individuals were contained per vial with those having more than 10 per vial (average = 20) shows no indication of an effect of crowding. Means for time of death of males uncrowded and crowded, are identical with the mean given above for the whole group with insignificant differences in standard errors. For females, mean survival time, in days with standard error 10.53 ± .50, was actually shorter in uncrowded vials as compared with the same computations in the larger groups, 11.63 ± .16. Differences between these means are not significant and the higher value for larger groups of females can be explained by chance inclusion of more of the longer lived wasps in the larger sample. It should be noted that even the trend is not what might be expected if adult population density is important in influencing the duration of life.

A second series of experiments at room temperature was performed with 100 females and 130 males of normal size kept in separate storage (10 per vial) from the time of their emergence as adults. No increased longevity was obtained for segregated females whether considered as a group or on the basis of extreme individuals. For segregated males a mean survival time in days of 8.38 ($s_{\bar{x}} =$ ± .26) indicates greater longevity.

Refrigerator temperature. At 4–5° C. the general principles (1) that larger animals live longer and (2) that females live longer than males of equal size were again demonstrated. Existence is greatly prolonged at lower temperatures as indicated by starved males which lived as long as 15 weeks and starved females which lived as long as 20 weeks. Using animals of normal size, 2.05 to 2.75 mm. long, the mean survival time with standard error for 193 males was $8.16 \pm .17$ weeks; for 109 females $9.71 \pm .39$ weeks.

Discussion

Comparisons of measurements of internal structures indicate that under starvation conditions the female differs from the male only by one major change, oösorption. Furthermore, in spite of smaller fat cells (Grosch, 1948c) females are shown to live longer than males of equal size. This fact is interesting in view of a recent publication (Georgiana, 1950) which reports longer life of females in honey fed culture. Taken together with present results these findings suggest that the stored materials obtained by oösorption are highly efficacious in maintaining life processes. Perhaps because of egg substance females contain more stored food *value* per unit storage volume. At any rate, the present results indicate that female abdomens are heavier. In a sense these views imply a different basis of metabolism for the sexes. In normal environment the female, through her diet, appears to have a different basis for metabolic processes. She ingests host body contents and although able to lay her oldest egg without this type of meal, it seems well founded that she must continue a caterpillar diet to insure continued egg production (Henschen, 1929) and circumvent oösorption (postulated on the basis of Flanders, 1942b). On the other hand the male shows interest in no food other than honey or syrup (Grosch, 1950). Furthermore, correlative with differences in the diet are demonstrable chemical differences between the feces of the different braconid sexes (Hase, 1922). Therefore, if the internal processes of the female normally involve substances in addition to those stored in the fat body, it seems a logical conclusion that the action systems are continued in starvation and cause oösorption. Thus revealed is mass disappearance of a substance in addition to fat body in confirmation of the authors' hypothesis that structural units of the fat body do not constitute the only depository for reserves in the female system.

While difference in type of metabolism is advanced as the explanation for differences in longevity between the sexes, difference in rate of metabolism is suggested in explanation of the correlation discovered between size and longevity within a sex. It is well known that smaller animals have a higher respiratory rate and that this rate is an index of metabolic activity (Heilbrunn, 1943).

Intimately associated with size differences in Habrobracon are differences in the size of microscopic structural units including fat cells and differences in the number of eggs produced (Grosch, 1948a). These situations are traceable to conditions during the larval feeding stage, the only period in the holometabolous life cycle during which the size increases (termed growth) occur. On this basis, crowding would be more likely to affect individual longevity if it occurred during the larval stage than at any other time during the Habrobracon life cycle. Furthermore, in contrast to the behavior of Drosophila, on which classic experiments in relation of population density to longevity have been made (see Hammond, 1938, 1939), stored adult Habrobracon adopt relatively quiescent attitudes in an undisturbed vial. The same pose and position may be maintained for hours or even weeks, depending upon the temperature. These facts could explain why results in an analysis based on group size demonstrated no noticeable effect of crowding on adult longevity.

An indication that segregated males live longer than males stored with females can be explained by the fact that mating preliminaries and the act of copulation involve excited active movements by the male (Grosch, 1948b). In the absence of mating stimuli these energy expending activities would not be indulged.

The bearing of the present results upon methods of investigation involving egg production and those pertaining to morphogenesis can be summed up quite briefly. The unfed adult should be employed in experiment or examined for phenotype during its first 48 hours, an adequate period of safety unless fat cells are under consideration. In the latter event examination should be made immediately upon emergence from cocoon as emphasized in a previous publication (Grosch, 1948c). Concerning comparisons of structural size, if genetic variation is under consideration, analysis should be made within sex types because Table I indicates slight sex differences in internal abdominal structures.

Studies on changes in microscopic anatomy are not typical of past trends in entomological literature. Starvation in adult insects has been approached chiefly from the standpoint of comparative longevity of unrelated species (Jackson, 1925; Maluf, 1939; Brues, 1946) or gross physiology of the intact organism (Uvarov, 1928; Wigglesworth, 1947). On the other hand, the amount of microscopic and histochemical observation made available through malnutrition and inanition experiments with vertebrates is impressively large, as indicated by consultation of almost any volume of Biological Abstracts. However, in histological detail, situations do not seem comparable and therefore literature on vertebrate changes are not reviewed herein.

Summary

1. In conditions of complete starvation, Habrobracon females live longer than males. This fact is more obvious at lower temperatures of storage.

2. There is a distinct positive correlation between size of a wasp and longevity. This is interpreted to have a basis in a lower metabolic rate in the larger organisms.

3. Weight loss is chiefly from the abdominal region which in the ultimate condition is extremely flattened dorsoventrally.

4. Histologically the greatest loss of material from both males and females is in number and size of the "fat" cells of the fat body. In females an additional loss occurs from the ovary, especially during the last stages of starvation. In wasps starved to the point of death the loss includes all the developing, as well as the mature eggs. Thus identified for the female seem to be two sources of reserve food supply. This point and attendant features are discussed from the standpoint of sex differences in structure, function and longevity.

5. Provided examination is made within the first two days, internal tissue other than the fat body should be in adequate quantitative state regardless of conditions of culture during the adult stage. It is also indicated that investigations employing ova should be started before two days after emergence from cocoons have elapsed.

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